

Methicillin Resistance among *Staphylococcus aureus* Isolates from Saudi Hospitals

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Key Words

Methicillin-resistant *Staphylococcus aureus* · Antimicrobial resistance · Nosocomial infection

Abstract

Objective: To determine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains among clinical isolates collected from the 4 tertiary hospitals in Makkah, Saudi Arabia, and to test the antimicrobial susceptibility patterns of *S. aureus* isolates against 9 antimicrobial agents. **Materials and Methods:** A total of 512 *S. aureus* clinical isolates were collected during a period of 1 year starting in April 2003 in Al-Noor, King Abdul-Aziz, Hera and King Faisal Hospitals, Makkah, Saudi Arabia. The sensitivity patterns of these isolates were determined using the Kirby-Bauer disk diffusion method. **Results:** The prevalence of MRSA among *S. aureus* isolates was 38.9% (199/512). Among 199 MRSA isolates, 78.8% showed multidrug resistance to erythromycin, gentamicin and oxytetracycline. **Conclusion:** The rate of MRSA resistance in this study was much higher than what had been reported in other areas of Saudi Arabia emphasizing the need for local or country-based surveillance to characterize and monitor MRSA and to develop strategies that will improve MRSA treatment and control.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide. There is a wide range in the prevalence of MRSA strains between different countries and even between hospitals in the same country. The extent of the spread of these organisms from hospital to hospital also shows variation [1]. Various studies documented increased costs associated with MRSA infection as well as the importance of colonization pressure [2, 3]. A recent US study [4] found that primary nosocomial bacteremia due to MRSA resulted in an approximately threefold increase in direct hospitalization costs when compared with those due to methicillin-susceptible *S. aureus*. There are several mechanisms responsible for methicillin resistance. The most important is the production of the penicillin-binding protein PBP2a encoded by the *mecA* gene. In addition, hyperproduction of β -lactamase and modified drug affinities of the usual PBPs are considered as minor resistant mechanisms [1, 5, 6]. Besides their resistance to all β -lactam antibiotics, MRSA strains may be resistant to several other classes of antibiotics, including the aminoglycosides, quinolones, clindamycin and erythromycin [5, 6]. Therefore, infections caused by these resistant strains are serious and difficult to treat.

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1011-7571/06/0151-0052\$23.50/0

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Table 1. Antibiotics sensitivity results of 9 antibacterial agents against 512 *S. aureus* (MRSA and MSSA) clinical isolates

Antibiotics	Resistance, %	
	MRSA (n = 199)	MSSA (n = 313)
Penicillin	100	95.5
Ampicillin	100	83
Oxacillin	100	0
Erythromycin	84.9	13.7
Cephalothin	100	4.7
Gentamicin	84.9	9.7
Oxytetracycline	90	18.5
Trimethoprim-sulfamethoxazole	80	8.8
Vancomycin	0	0

MSSA = Methicillin-sensitive *S. aureus*.

The aim of this study was to determine the prevalence of MRSA strains isolated from nosocomial infections and their antimicrobial resistant patterns in the 4 tertiary hospitals in Makkah, Saudi Arabia.

Materials and Methods

S. aureus Isolates

From April 2003 to March 2004, a total of 512 nonreplicate *S. aureus* clinical isolates from hospitalized patients were collected from the 4 tertiary hospitals Al-Noor (560 beds), King Abdul-Aziz (272 beds), Hera (263 beds) and King Faisal (221 beds) in Makkah (124–132 samples/hospital). Infections were defined as hospital acquired when the patient had been hospitalized for more than 48 h [7]. The identity of these isolates was confirmed using colonial morphology on blood agar plates (Oxoid, UK), Gram stain and positive catalase and coagulase tests (Murex Diagnostic Ltd., UK).

Antibiotic Susceptibility Testing

Susceptibility testing was performed with the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates (Oxoid) using NCCLS guidelines [8]. Briefly, the tests were performed by preparing a high-density inoculum made by diluting 5 colonies grown overnight on Columbia agar supplemented with 5% sheep blood (Oxoid) in 5 ml of Muller-Hinton broth (Oxoid) to prepare a suspension equivalent in density to 0.5 McFarland barium sulfate standard unit. The entire surface of the Mueller-Hinton agar plate was covered with the required inoculum, and the plate was dried for 5 min before the antibiotic disks were placed on the surface and incubated for 18–24 h at 37°C. Following the incubation period, the sensitivity results were determined by comparing the diameter of the zones of growth inhibition with NCCLS standards [8]. The antibiotics tested (Oxoid) were gentamicin (10 µg), erythromycin (15 µg), vancomycin (30 µg), penicillin (10 units), ampicillin (10 µg), cephalothin (30 µg), oxytetracycline (30 µg) and tri-

Table 2. Distribution of clinical specimens from which MRSA was isolated

Sample source	Isolates
Wound	70 (35.2%)
Respiratory tract	53 (26.6%)
Blood	53 (26.6%)
Urine	10 (5%)
Ear	9 (4.5%)
Eye	4 (2.1%)
Total	199 (100%)

methoprim-sulfamethoxazole (1.25/23.75 µg). *S. aureus* ATCC 25923 was used as a control strain.

Isolates were tested for methicillin resistance by the Kirby-Bauer disk diffusion method as described above using an oxacillin (1 µg) disk (Oxoid) on Mueller-Hinton agar supplemented with 4% NaCl and incubated at 35°C for 24 h. A zone of inhibition of 13 mm or more was considered as oxacillin sensitive [9]. Methicillin resistance results were confirmed by the MRSA screen test (Denka Seiken Co. Ltd., Tokyo, Japan) which is based on the agglutination of latex particles sensitized with monoclonal antibodies against PBP2a. This test was used according to the manufacturer's instructions.

The resistance rate was calculated as the number of intermediate and resistant isolates divided by the total number of isolates. Multidrug resistance was defined as resistance to penicillin and oxacillin plus 3 or more of the following agents: erythromycin, clindamycin, gentamicin and oxytetracycline [9].

Results

Of the 512 *S. aureus* strains tested, 199 (38.9%) contained PBP2a and were methicillin resistant according to the disk diffusion test. Similar results were found with the latex agglutination test which further confirmed MRSA results. Rates of resistance of methicillin-sensitive *S. aureus* and MRSA to the other antibiotics tested are shown in table 1. Of 199 MRSA isolates, 157 (78.9%) were multidrug resistant. The prevalences of MRSA in Al-Noor, King Abdul-Aziz, Hera and King Faisal Hospitals were 41.5, 37.5, 40.3 and 36.3%, respectively, thereby indicating no significant difference in the prevalence of MRSA among the 4 tertiary hospitals.

Infections with MRSA were more common among males (59%) and among older (≥ 55 years) than female and younger patients. Among the 199 MRSA strains, 70 (35.2%) were isolated from skin wounds, 53 (26.6%) from

respiratory samples, 53 (26.6%) from blood, 10 (5%) from urine, 9 (4.5%) from eye swabs and 4 (2.1%) from ear swabs (table 2).

Discussion

Only 2 years after methicillin had been introduced in 1959, the first MRSA was described, and the first nosocomial MRSA epidemic was reported soon afterwards [10]. Since the introduction of methicillin for clinical use, the proportion of MRSA strains isolated worldwide has risen sharply [11–13]. For example in the USA, the rate of MRSA isolated has risen from 2% in 1975 to 35% in 1996 [11]. In Japan, 60% of *S. aureus* isolated were resistant to methicillin among 7,000 strains collected between 1992 and 1993 [12]. In England and Wales, the number of hospitals affected by epidemic MRSA (EMRSA-15 or -16) has increased from about 40 a month in 1993 to over 110 in March 1996 [13]. MRSA therefore has become a major global nosocomial pathogen, against which vancomycin is the recommended therapeutic agent. Antibiotic abuse and poor infection control, and not increased virulence and transmissibility, have been blamed for the spread of this organism [14].

The incidence of MRSA strains is particularly high in Japan [12], being 60% nationwide compared to 35% in the USA [11]. In 2001, the rates of MRSA in European countries were found to be 28% in the UK, 0.8% in Denmark, 17.5% in Germany, 34% in Greek hospitals, 43–58% in Italy and 54% in Portugal [15]. In Saudi Arabia, the prevalence of MRSA strains has ranged from 7.5 to 33% [16–18]. These variations among different countries can be affected by variations in patient populations, hospital care practices, infection control activities, the time of study and the biological characteristics of the *Staphylococcus* strains.

In this study, the rate of resistance (38.9%) of *S. aureus* is much higher than what has been reported by researchers in other regions of Saudi Arabia as well as in many other studies [18–23]. Several reasons may account for the high rate of resistance among *S. aureus* in this study; these include misuse of antibiotics, prescription of most antibiotics without minimal diagnostic procedures, prescription of antibiotics without restriction guidelines and lack of or inadequate knowledge of infectious diseases and proper antimicrobial usage.

All MRSA isolates in this study were sensitive to vancomycin, but a large proportion of our isolates (78.8%) showed multidrug resistance to erythromycin, gentami-

cin and oxytetracycline. Similar findings have been reported elsewhere [23].

The finding that skin and soft tissue infections were the most common sites of culture for MRSA in our study is in agreement with prior investigations [23–25].

Conclusion

The rate of MRSA resistance in this study is much higher than what has been reported in other areas of Saudi Arabia and many other international countries emphasizing the need for local or country-based surveillance to characterize and monitor MRSA and to develop strategies that will improve MRSA treatment and control.

Acknowledgement

The authors gratefully acknowledge the help and support of: Dr. Ossam F. Al-Bar, Dean of the Custodian of the Two Holy Mosques Institute of Hajj Research; Dr. Ahmed Ashi, Laboratory Director, Hera Hospital; Dr. Yousif Khodri, Laboratory Director, Al-Noor Hospital; Mr. Faiz Hafiz, Laboratory Director, King Faisal Hospital; Mr. Adel Ashi, Laboratory Specialist, King Abdul-Aziz Hospital; Mr. Abdulsamad A. Bin-rag, Mr. Ahmad S. Ga-asher and Mr. Ala'a Kaki.

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